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Biological

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Comparison of Stem-cell-mediated Osteogenesis and Dentinogenesis

ABSTRACT

The difference between stem-cell-mediated bone and dentin regeneration is not yet well-understood. Here we use an *in vivo* stem cell transplantation system to investigate differential regulation mechanisms of bone marrow stromal stem cells (BMSSCs) and dental pulp stem cells (DPSCs). Elevated expression of basic fibroblast growth factor (bFGF) and matrix metalloproteinase 9 (MMP-9, gelatinase B) was found to be associated with the formation of hematopoietic marrow in BMSSC transplants, but not in the connective tissue of DPSC transplants. The expression of dentin sialoprotein (DSP) specifically marked dentin synthesis in DPSC transplants. Moreover, DPSCs were found to be able to generate reparative dentin-like tissue on the surface of human dentin *in vivo*. This study provided direct evidence to suggest that osteogenesis and dentinogenesis mediated by BMSSCs and DPSCs, respectively, may be regulated by distinct mechanisms, leading to the different organization of the mineralized and non-mineralized tissues.

KEY WORDS: bone marrow stromal stem cell, dental pulp stem cell, osteoblast, odontoblast, transplantation.

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INTRODUCTION

Adult bone marrow stromal stem cells (BMSSCs) and adult dental pulp stem cells (DPSCs) are multipotent stem cells capable of differentiating into various cell types, including, but not limited to, osteoblasts/odontoblasts, adipocytes, and neural cells (Prockop, 1997; Azizi *et al.*, 1998; Gronthos *et al.*, 2000, 2002; Bianco *et al.*, 2001). Previous studies also demonstrated that BMSSCs are able to differentiate into chondrocytes and muscle cells (Prockop, 1997; Ferrari *et al.*, 1998; Johnstone *et al.*, 1998). Although bone and dentin are similar in their matrix protein composition, their organ structures are totally different. One of the most striking characteristics is that BMSSCs and DPSCs can generate a bone/marrow organ structure and a dentin/pulp complex, respectively (Krebsbach *et al.*, 1997; Gronthos *et al.*, 2000). However, the detailed mechanisms involved in the initiation and maintenance of the bone/marrow organ and dentin/pulp complex have yet to be determined. In this study, we demonstrate that bFGF and MMP-9, two important angiogenic factors, are temporally expressed in the connective tissue compartment of BMSSC transplants prior to marrow formation. In contrast, dentin sialoprotein (DSP), a highly specific dentin protein, is highly expressed during dentinogenesis in the DPSC transplants. Furthermore, we found that DPSCs were capable of forming reparative dentin-like tissue on the surface of human dentin *in vivo*. This study suggests that BMSSCs and DPSCs use distinct regulatory mechanisms to control their *in vivo* tissue regeneration.

MATERIALS & METHODS

Subjects and Cell Culture

Bone marrow mononuclear cells were purchased from Poietic Technologies (Gaithersburg, MD, USA). Human impacted third molars were collected from adults (19-29 yrs of age) at the Dental Clinic of the National Institute of Dental & Craniofacial Research under a protocol approved by the NIH Office of Human Subjects Research. Human DPSCs were isolated and cultured as previously described (Gronthos *et al.*, 2000, 2002). Briefly, the pulp tissue was separated from the crown and root and then digested in a solution of 3 mg/mL collagenase type I (Worthington Biochem, Freehold, NJ, USA) and 4 mg/mL dispase (Boehringer Mannheim GmbH, Mannheim, Germany) for 1 hr at 37°C. Single-cell suspensions (from 0.01 to 1 x 10^5/well) of bone marrow and dental pulp were cultured in six-well plates (Costar, Cambridge, MA, USA) with alpha modification of Eagle's Medium (GIBCO BRL, Grand Island, NY, USA) supplemented with 15% fetal calf serum (Equitech-Bio Inc., Kerrville, TX, USA), 100 μM L-ascorbic acid 2-phosphate (WAKO, Tokyo, Japan), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Biofluids Inc., Rockville, MD, USA), then incubated at 37°C in 5% CO_2.

Transplantation

Approximately 4.0 x 10^6 of *ex vivo* expanded BMSSCs and DPSCs were mixed with 40 mg of hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic powder

(Zimmer Inc., Warsaw, IN, USA) and then incubated at 37°C for 2 hrs. After centrifugation at 1500 rpm for 7 min, the supernatant was removed. The cell pellets with HA/TCP were transplanted into 10-week-old immunocompromised beige mice (NIH-bg-nu-xid, Harlan Sprague-Dawley, Indianapolis, IN, USA) as previously described (Krebsbach *et al.*, 1997; Gronthos *et al.*, 2000). Midlongitudinal skin incisions were made on the dorsal surface of each mouse, and subcutaneous pockets were made by blunt dissection. A single transplant was placed in each pocket, with up to 4 transplants *per* animal. These procedures were performed in accordance with specifications of an NIDCR/NIH IRB-approved animal protocol (NIDCR #02-222). The transplants were recovered at 2, 4, 8, and 16 wks post-transplantation, fixed with 4% formalin, decalcified with buffered 10% EDTA (pH 8.0), and then embedded in paraffin. For DPSC/dentin transplantation, the roots of the third molars were cut to expose the pulp chamber, a thin layer of pulpal dentin surface was removed by means of a carbide bur, and the exposed surface was treated with 1% acetic acid for 10 min at room temperature and then washed three times with PBS. Approximately 2.0 x 10^6 DPSCs were loaded onto the acid-treated/PBS-washed dentin surface and incubated under the cell culture medium at 37°C for 12 hrs. The root foramen was sealed with Gelfoam (absorbable gelatin sponge, Pharmacia & Upjohn Company, Kalamazoo, MI, USA), and the culture medium was removed before the transplantation. The DPSC/dentin complexes were then transplanted subcutaneously. Acid-treated dentin samples cultured with BMSSC (2.0 x 10^6) were transplanted as controls.

In situ Hybridization

A human-specific *alu* probe labeled with digoxigenin was used for *in situ* hybridization. The probe was prepared by PCR containing 1x PCR buffer (Perkin Elmer, Foster City, CA, USA), 0.1 mM dATP, 0.1 mM dCTP, 0.1 mM dGTP, 0.065mM dTTP, 0.035 mM digoxigenin-11-dUTP, 10 pmol of specific primers, and 100 ng of human genomic DNA as template. Primers for *alu* (GenBank Accession Number X53550) included sense, 5′-TGGCTCA-CGCCTGTAATCC-3′ (base number 90-108), and antisense, 5′-TTTTTTGAGACGGAGTCTCGC-3′ (base number 344-364). Unstained sections were deparaffinized and hybridized with the digoxigenin-labeled *alu* probe by means of the mRNAlocator-Hyb Kit (Cat. #1800; Ambion, Inc., Austin, TX, USA).

Immunohistochemistry

The BMSSC and DPSC transplants were fixed, decalcified, and embedded in paraffin, and 5-micron tissue sections were incubated with the primary antibodies at room temperature for 1 hr. Primary antibodies used were against: bFGF, MMP-9 (1:200 dilution; rabbit polyclonal, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); mitochondria (1:100 dilution; rabbit anti-human-specific, Chemicon, Temecula, CA, USA); and dentin sialoprotein (1:200 dilution of LF-151) (Gronthos *et al.*, 2002). Histostain SP Kits were used for biotinylated second antibodies and HRP enzyme-avidin conjugate incubation according to the manufacturer's instructions (Zymed Laboratories Inc., South San Francisco, CA, USA).

RESULTS

Generation of Ectopic Bone/Marrow and Dentin/Pulp Organs

At 2 wks post-transplantation, most of the BMSSC and DPSC transplants (7 out of 9 for BMSSC and 7 out of 10 for DPSC) had not yet generated mineralized tissues (Figs. 1A, 1E). Only

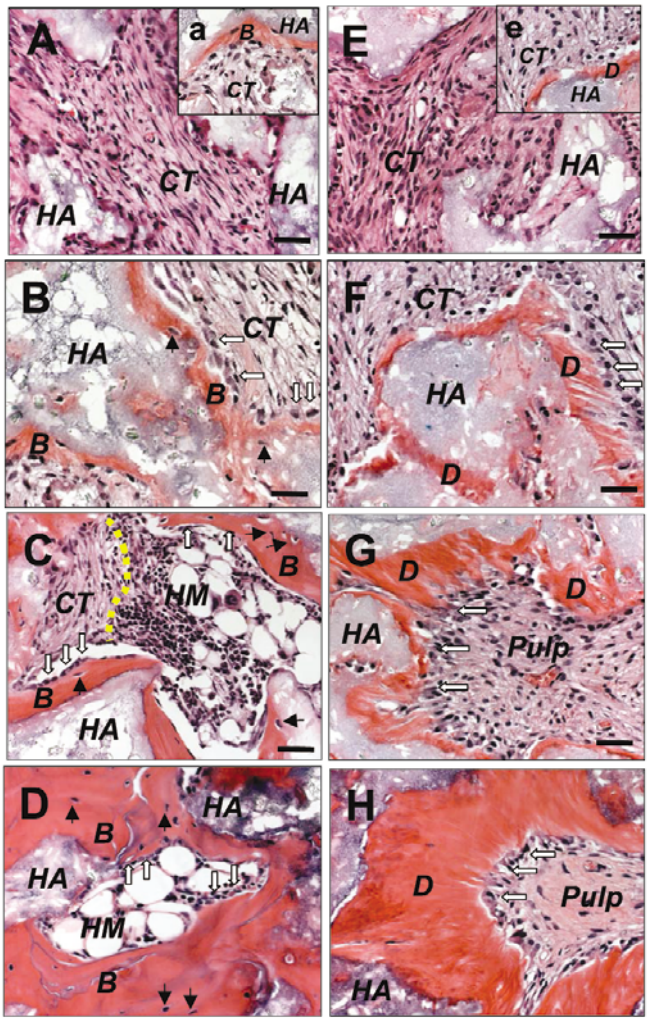

Figure 1. H&E staining of BMSSC and DPSC transplants. **(A and a)** BMSSC transplant at 2 wks post-transplantation showed connective tissue (*CT*) surrounding the HA/TCP carriers (*HA*). Bone regeneration (*B*) on the HA/TCP (*HA*) was found only on a very limited number of transplants. **(B)** At 4 wks post-transplantation, BMSSCs were able to generate bone (*B*) on the surface of the HA/TCP (*HA*) and had differentiated into osteoblasts (open arrows) and osteocytes (black arrows). **(C)** At 8 wks post-transplantation of the BMSSC transplant, a bone/marrow organ-like structure was generated, where osteoblasts (open arrows) lined the surface of bone tissue containing osteocytes (black arrows), and the connective tissue (*CT*) was replaced by hematopoietic marrow (*HM*). The interface between the hematopoietic marrow and connective tissue is indicated (yellow dashed line). **(D)** At 16 wks post-transplantation, the BMSSC transplant had developed into a mature bone/marrow organ, in which a significant amount of bone (*B*) was generated along with hematopoietic marrow elements (*HM*). **(E and e)** The DPSC transplant at 2 wks post-transplantation showed connective tissue (*CT*) surrounding the HA/TCP (*HA*). Similar to the BMSSC transplants, only very few transplants showed dentin (*D*) formation on the surface of the HA/TCP (*HA*). **(F)** After 4 wks of transplantation, DPSCs differentiated into odontoblasts (open arrows) responsible for new dentin (*D*) formation on the surface of the HA/TCP (*HA*). **(G)** At 8 wks post-transplantation of the DPSC transplant, a dentin/pulp complex was generated, at which odontoblasts (open arrows) lined the surface of the newly formed dentin (*D*), and the pulp-like tissue (*Pulp*) contained blood vessels and connective tissue. **(H)** At 16 wks post-transplantation, the DPSC transplant had continued to develop into a dentin/pulp complex, in which a significant amount of dentin (*D*) was generated adjacent to the pulp-like tissue (*Pulp*). Bar, 40 μm.

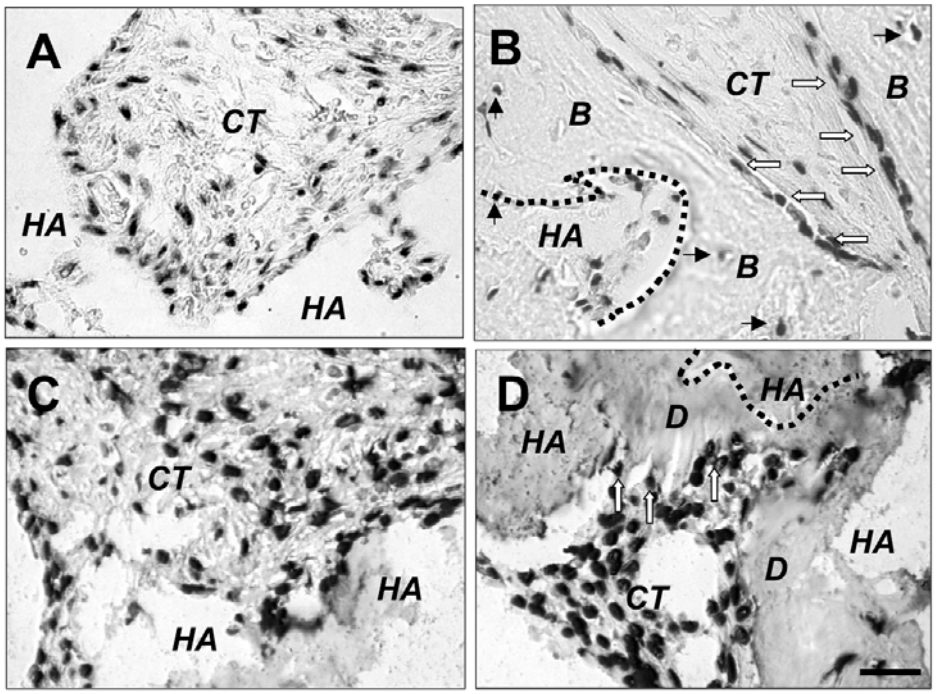

Figure 2. Human *alu in situ* hybridization of BMSSC and DPSC transplants. **(A)** Human BMSSCs (dark nuclear staining) were either attached to the surface of the HA/TCP (*HA*) or in the connective tissue compartment (*CT*) at 2 wks post-transplantation. **(B)** After 8 wks' transplantation, BMSSCs differentiated into osteoblasts (open arrows) and osteocytes (black arrows) to generate bone (*B*) on the surface of the HA/TCP (*HA*). The number of BMSSCs in the connective tissue compartment (*CT*) was significantly diminished. **(C)** Human DPSCs (dark nuclei staining) either attached to the surface of the HA/TCP (*HA*) or resided in the connective tissue compartment (*CT*) at 2 wks post-transplantation. **(D)** At 8 wks post-transplantation, DPSCs differentiated into odontoblasts (open arrows) to generate dentin (*D*) on the surface of the HA/TCP (*HA*). The number of DPSCs present in the connective tissue compartment (*CT*) is comparable with the number present at 2 wks post-transplantation. Bar, 20 μm.

approximately 20-30% of the transplants (2 out of 9 for BMSSC and 3 out of 10 for DPSC) showed a minimal amount of bone or dentin formation (Figs. 1a, 1e). At 4 wks post-transplantation, BMSSCs and DPSCs differentiated into osteoblasts and odontoblasts and generated bone and dentin, respectively, on the surface of the HA/TCP in all transplants (Figs. 1B, 1F). At 8 wks post-transplantation, BMSSCs generated a bone/marrow organ structure in which abundant bone tissue was formed and hematopoietic marrow elements replaced the connective tissue (Fig. 1C). In contrast, DPSCs generated a dentin/pulp-like complex where the pulp-like tissue was comprised of fibrous connective tissue, blood vessels, and odontoblasts associated with newly formed dentin (Fig. 1G). Moreover, transplanted BMSSCs and DPSCs continued to generate bone and dentin at least until 16 wks post-transplantation, at which time they formed a mature bone/marrow organ structure and dentin/pulp complex, respectively (Figs. 1D, 1H). The transplanted human BMSSCs and DSPCs were found either attached to the surface of the HA/TCP carrier or in the connective tissue compartment 2 wks post-transplantation (Figs. 2A, 2C). Most of the surviving BMSSCs differentiated into either osteoblasts or osteocytes, and the number of BMSSCs present in the connective tissue compartment decreased with time (Fig. 2B). However, DPSCs not only differentiated into odontoblasts that remained directly associated with the contiguous transplant carrier HA-dentin matrix, but also continued to reside in the connective tissue compartment even 8 wks post-transplantation (Fig. 2D).

Establishment of Connective Tissue Compartments in Ectopic Organs

To examine the potential mechanism of hematopoietic marrow formation, we used immunohistochemical staining to show that bFGF and MMP-9 are temporally expressed in the connective tissue compartment prior to the generation of hematopoietic marrow elements in BMSSC transplants. At 2 wks post-transplantation, only a limited number of cells expressed bFGF in the connective tissue compartment (Fig. 3A). Following bone formation at 4 wks post-transplantation, the number of cells expressing bFGF was significantly higher (Fig. 3B). This high level of bFGF expression continued until the connective tissue was replaced by hematopoietic marrow elements at 8 wks post-transplantation, at which point the level of bFGF expression was significantly diminished (Figs. 3C, 3D). There was no bFGF expression in DPSC transplants at any time point, and therefore bFGF appeared not to play an important role in the formation of the dentin/pulp-like complex (Figs. 3G, 3H).

The expression pattern of MMP-9 was somewhat similar to the expression of bFGF in BMSSC transplants. There was no MMP-9 expressed in BMSSC transplants 2 wks post-transplantation (Fig. 3E); however, it was highly expressed in the connective compartment of BMSSC transplants after 4 wks (Fig. 3F), and this elevated expression significantly diminished with the formation of hematopoietic marrow elements (data not shown). Like bFGF, MMP-9 was not seen in the dentin/pulp complex of the DPSC transplants. VEGF was found to be equally expressed in BMSSC and DPSC transplants, along with newly formed blood vessels (Figs. 3I, 3J).

Dentinogenesis of DPSCs

To assess the potential contribution of DSP to dentinogenesis, we examined the expression of DSP in dentinogenic cells of DPSC transplants and osteogenic cells of BMSSC transplants. Dentinogenic cells, similar to odontoblasts in human dental pulp, clearly showed a positive immunostaining for DSP antibody (Figs. 4A, 4C). DSP was not detectable in osteogenic cells of human bone and BMSSC transplants by immunohistochemical staining (Figs. 4B, 4D).

To examine further the tissue regeneration capability of BMSSCs and DPSCs, we transplanted BMSSCs and DPSCs using human dentin as a carrier. BMSSCs failed to form

mineralized tissue on the surface of human dentin and failed to induce pulp-like connective tissue (Fig. 4E). In contrast, DPSCs were capable of generating a reparative dentin-like structure directly on the surface of human dentin (Fig. 4F). Like reparative dentin formation in the human pulp-dentin system, the newly formed dentin did not contain an organized dentinal tubule structure, a result different from that shown in DPSC/HA/TCP transplants (Fig. 1G). The reparative dentin-like structure was initiated on the acid-treated human dentin surface that provided a scaffold for the dentinogenesis of DPSCs. Newly formed tissue was generated by dentinogenic cells located on its surface and cells trapped inside the tissue (Fig. 4F). To characterize the newly regenerated reparative dentin-like tissue on the pre-existing human dentin surface, we used immunohistochemical staining to show that the pre-existing dentin scaffold and the dentinogenic cells responsible for forming reparative dentin-like tissue were positive for DSP antibody staining (Fig. 4G). Connective tissue failed to show immunopositive staining for DSP antibody (Figs. 4G, 4H). Interestingly, only the peritubular dentin structure of the pre-existing dentin scaffold was immunoreactive to DSP antibody (Figs. 4G, 4H). Therefore, our inability to detect DSP expression on the matrix of newly formed reparative dentin-like tissue that clearly failed to show tubular dentin structure was not surprising (Figs. 4F, 4G). We used human-specific anti-mitochondrial immunohistochemistry to identify human cells and their patterns of distribution in the DPSC/dentin transplants. It was found that the dentinogenic cells either lining the surface of or trapped within newly formed reparative dentin-like structure were human in origin (Figs. 4I, 4J).

DISCUSSION

Although the stem cell transplantation system for osteogenesis and dentinogenesis used in this study has been established previously (Krebsbach *et al.*, 1997; Gronthos *et al.*, 2000), the dynamic process of tissue regeneration and the distinct mechanisms of tissue regeneration mediated by BMSSCs and DPSCs are not yet elucidated. Osteogenesis and dentinogenesis begin at 2-4 wks post-transplantation, and eventually lead to the regeneration of a bone/marrow organ structure and a dentin/pulp-like complex in BMSSC and DPSC transplants, respectively. By 16 wks post-transplantation, the transplants show even more formation of mineralized tissue when compared with eight-week transplants, indicating that BMSSCs and DPSCs have the potential to continue forming mineralized tissue after the organ-like structures are formed. This also suggests that both BMSSCs and DPSCs are not only able to differentiate into osteoblasts/odontoblasts *in vivo* early in the transplantation process, but also are capable of inducing host cells to participate in tissue regeneration by the formation of a hematopoietic marrow and a pulp-like complex. The connective tissue compartment aids osteogenesis by supporting blood vessel ingrowth and subsequent hematopoietic marrow formation in BMSSC transplants. Blockage of the blood vessel supply, for example, can inhibit BMP-induced ectopic bone formation *in vivo* (Mori *et al.*, 1998).

Development of stem-cell-mediated organ-like structure is a dynamic process that involves mutual interactions between transplanted stem cells and the host microenvironment. Eventually, the organ-like structure is a chimeric structure of

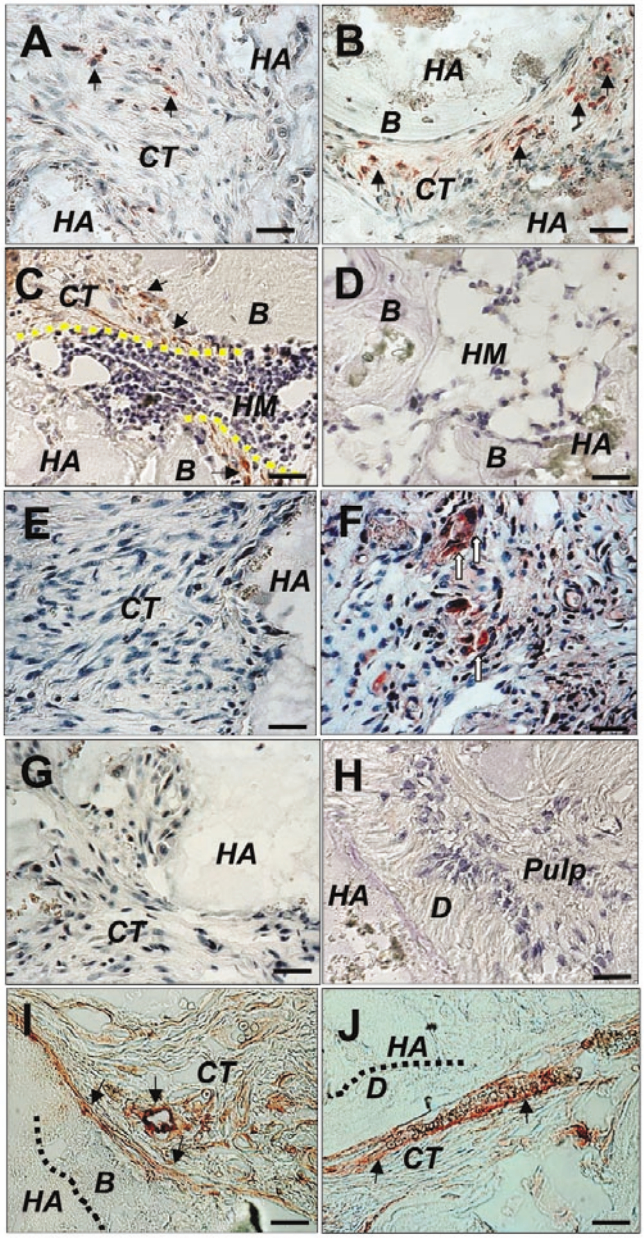

Figure 3. Immunohistochemical staining of bFGF and MMP-9 in BMSSC transplants. **(A)** BMSSC transplants showed that some cells were immunopositive for bFGF antibody staining (black arrows) in the connective tissue compartment (*CT*) surrounding the HA/TCP (*HA*) at 2 wks post-transplantation. **(B)** After 4 wks' BMSSC transplantation, along with new bone (*B*) formation, the number of bFGF-positive cells (black arrows) was increased in the connective tissue compartment (*CT*). **(C)** After 8 wks' BMSSC transplantation, the connective tissue (*CT*) showed a high-level expression of bFGF (black arrows). Newly formed hematopoietic marrow showed a negative staining for bFGF antibody staining. The interface between the hematopoietic marrow and connective tissue is indicated (yellow dashed line). **(D)** When hematopoietic marrow (*HM*) completely replaced the connective tissue at 16 wks post-transplantation, a very limited number of cells expressed bFGF in BMSSC transplants. **(E)** BMSSC transplant showed a negative staining for MMP9 antibody at 2 wks post-transplantation. **(F)** After 4 wks' transplantation, MMP9 was expressed in the connective tissue compartment of BMSSC transplants (open arrows). **(G and H)** DPSC transplants showed a negative staining of bFGF prior to dentin regeneration at 2 wks post-transplantation (G) and after dentin formation at 8 wks post-transplantation (H). **(I and J)** VEGF was equally expressed on blood vessels of BMSSC (I) and DPSC (J) transplants at 4 wks post-transplantation (black arrows). The black dashed lines represent interfaces between HA/TCP and newly formed bone (I) or dentin (J). Bar, 20 μm.

donor and host cellular components. Here we show that bFGF, an important mitogen for vascularization and organogenesis (Bouma-ter Steege *et al.*, 2001; Traver and Zon, 2002), is specifically up-regulated and may play an important role in not only ingrowth of blood vessels, but also the recruitment of hematopoietic elements in BMSSC transplants. MMP-9, an endothelial-cell-secreted matrix metalloproteinase (gelatinase B), is well-known for its ability to degrade collagen present in the vascular basement membrane (Murphy and Crabbe, 1995), and it plays a central role during angiogenesis (Nguyen *et al.*, 2001). It is reasonable to speculate that up-regulated MMP-9 expression in BMSSC transplants contributes to angiogenesis and subsequent hematopoietic marrow formation. These processes are absent in DPSC transplants, although vascularization can be demonstrated by VEGF expression.

While DSP has been reported to be present not only in dentin but also in bone at very low levels (Qin *et al.*, 2002), in our experiments it was highly expressed in the newly formed dentin but could not be detected by immunohistochemical staining in osteoblasts and bone matrix of BMSSC transplants (Gronthos *et al.*, 2002). Although the mechanisms of how DSP contributes to dentinogenesis in DPSC transplants are not yet clear, it may be involved in the generation of a dentin/pulp complex *in vivo*.

Dentin does not remodel, but responds to an injury by forming reparative dentin to protect the dental pulp (Murray *et al.*, 2000; About *et al.*, 2001). It has been reported that bone sialoprotein and dentin matrix components are able to stimulate reparative dentin formation (Decup *et al.*, 2000; Smith and Lesot, 2001) and that growth factors such as TGFβs and BMP-7 are capable of stimulating secretion of extracellular matrix by odontoblasts (Sloan and Smith, 1999; Sloan *et al.*, 2000). However, the restoration of damaged dentin is still limited to conventional treatments such as fillings and crown restorations (Baum and Mooney, 2000; Kaigler and Mooney, 2001). Thus, regeneration of living teeth, or parts thereof, is considered to be a promising new therapeutic strategy. Unlike in the DPSC/HA/TCP transplants (Gronthos *et al.*, 2000, 2002), DPSCs failed to generate an organized dentinal tubule structure in the DPSC/dentin transplants. This may be due to differences in the chemical composition and three-dimensional structure of HA/TCP and acid-treated dentin. Here we demonstrate that DPSCs are capable of forming reparative dentin-like structure on the surface of human dentin, indicating the possibility and challenge of using DPSCs to repair tooth structures and to seal root canals.

The mechanisms that control the distinct differentiation of BMSSCs and DPSCs *in vivo* are not fully understood at this time. This study provides the first evidence to demonstrate that

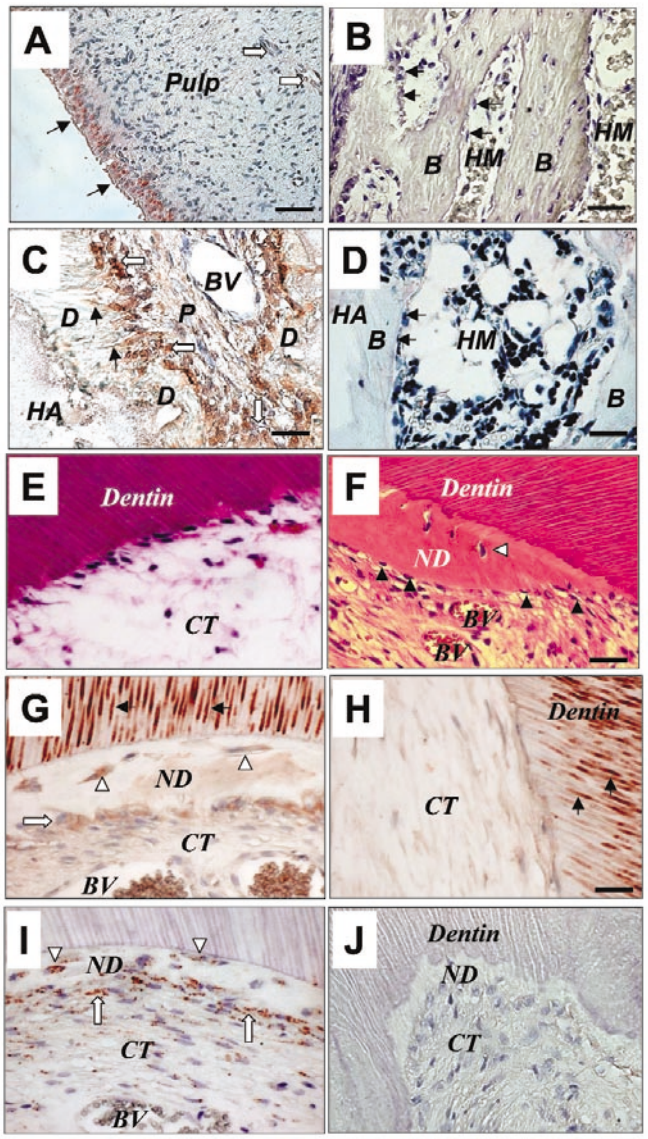

Figure 4. Characterization of DPSC-mediated dentinogenesis *in vivo*. **(A)** Odontoblasts of human dental pulp show a positive immunostaining for DSP antibody (black arrows). Other cells and blood vessels (open arrows) within the dental pulp are negative to DSP antibody staining. **(B)** There is no DSP antibody immunostaining on human bone (*B*), osteoblasts (black arrows), and hematopoietic marrow elements (*HM*). **(C)** DPSC transplant at 8 wks post-transplantation. There is DSP-positive immunostaining on dentinogenic cells (open arrows) and tubular dentin (black arrows). Pulp-like tissue (*P*) contains blood vessel (*BV*) and connective tissue. Dashed line shows the interface between the hydroxyapatite carrier (*HA*) and the newly generated dentin (*D*). **(D)** At 8 wks post-transplantation, BMSSCs differentiated into osteoblasts (black arrows) to generate bone (*B*) and induced hematopoietic marrow formation (*HM*). There was no DSPP antibody immunostaining on newly formed bone and osteogenic cells. **(E)** BMSSCs were loaded onto the acid-treated dentin and cultured for 12 hrs. Then BMSSC/dentin complexes were transplanted into immunocompromised mice. There was no mineralized tissue regeneration after 8 wks' transplantation. **(F)** Newly formed reparative dentin-like structure (*ND*) attached to the surfaces of human dentin in DPSC/dentin transplants. There were blood vessels (*BV*) and connective tissue (*CT*) associated with dentinogenic cells (black arrows). DPSCs were unable to generate organized tubular dentin structure; instead, they formed reparative dentin-like structure containing entrapped cells (triangle open arrows). **(G)** In DPSC/dentin transplants, dentinogenic cells (open arrows) and trapped cells (open triangles) within the newly formed reparative dentin-like structure (*ND*) were immunoreactive to human DSP antibody. Human dentin scaffold (*Dentin*) showed a positive DSP immunostaining on peritubular structures (black arrows). Connective tissue (*CT*) contained blood vessels (*BV*). **(H)** DSP immunohistochemical staining of BMSSC/dentin transplant showed a positive staining on the peritubular dentin (black arrows). Connective tissue (*CT*) showed a negative immunostaining for DSP antibody. **(I)** Immunohistochemical staining of human-specific anti-mitochondria antibody showed that human DPSCs differentiated into dentinogenic cells lining the surfaces (open arrows) or trapped within (open triangles) the newly generated reparative dentin-like tissue (*ND*). DPSCs were also found within the connective tissue compartment (*CT*) at 8 wks post-transplantation. **(J)** Negative control of immunohistochemical staining on DPSC/dentin transplant with pre-immuno serum. Bar, 40 μm in panels A and B, 20 μm in C-J. E and F were stained with hematoxylin & eosin.

bFGF/MMP-9 and DSP may play important roles in the formation of a bone/marrow organ structure and a dentin/pulp complex, respectively, suggesting that osteogenesis and dentinogenesis are controlled by different mechanisms.

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REFERENCES

About I, Murray PE, Franquin JC, Remusat M, Smith AJ (2001). Pulpal inflammatory responses following non-carious class V restorations. *Oper Dent* 26:336-342.

Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ (1998). Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats—similarities to astrocyte grafts. *Proc Natl Acad Sci USA* 95:3908-3913.

Baum BJ, Mooney DJ (2000). The impact of tissue engineering on dentistry. *J Am Dent Assoc* 131:309-318.

Bianco P, Riminucci M, Gronthos S, Robey PG (2001). Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells* 19:180-192.

Bouma-ter Steege JC, Mayo KH, Griffioen AW (2001). Angiostatic proteins and peptides. *Crit Rev Eukaryot Gene Expr* 11:319-334.

Decup F, Six N, Palmier B, Buch D, Lasfargues JJ, Salih E, *et al.* (2000). Bone sialoprotein-induced reparative dentinogenesis in the pulp of rat's molar. *Clin Oral Investig* 4:110-119.

Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, *et al.* (1998). Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279:1528-1530.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA* 97:13625-13630.

Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, *et al.* (2002). Stem cell properties of human dental pulp stem cells. *J Dent Res* 81:531-535.

Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU (1998). In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* 238:265-272.

Kaigler D, Mooney D (2001). Tissue engineering's impact on dentistry. *J Dent Educ* 65:456-462.

Krebsbach PH, Kuznetsov SA, Satomura K, Emmons RV, Rowe DW, Robey PG (1997). Bone formation in vivo: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. *Transplantation* 63:1059-1069.

Mori S, Yoshikawa H, Hashimoto J, Ueda T, Funai H, Kato M, *et al.* (1998). Antiangiogenic agent (TNP-470) inhibition of ectopic bone formation induced by bone morphogenetic protein-2. *Bone* 22:99-105.

Murphy G, Crabbe T (1995). Gelatinases A and B. *Methods Enzymol* 248:470-484.

Murray PE, About I, Lumley PJ, Smith G, Franquin JC, Smith AJ (2000). Postoperative pulpal and repair responses. *J Am Dent Assoc* 131:321-329.

Nguyen M, Arkell J, Jackson CJ (2001). Human endothelial gelatinases and angiogenesis. *Int J Biochem Cell Biol* 33:960-970.

Prockop DJ (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 276:71-74.

Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, *et al.* (2002). The expression of dentin sialophosphoprotein gene in bone. *J Dent Res* 81:392-394.

Sloan AJ, Smith AJ (1999). Stimulation of the dentine-pulp complex of rat incisor teeth by transforming growth factor-beta isoforms 1-3 in vitro. *Arch Oral Biol* 44:149-156.

Sloan AJ, Rutherford RB, Smith AJ (2000). Stimulation of the rat dentine-pulp complex by bone morphogenetic protein-7 in vitro. *Arch Oral Biol* 45:173-177.

Smith AJ, Lesot H (2001). Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? *Crit Rev Oral Biol Med* 12:425-437.

Traver D, Zon LI (2002). Walking the walk: migration and other common themes in blood and vascular development. *Cell* 108:731-734.